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(54) Title: DERIVATIZED TRIS-CATECHOL CHELATING AGENTS

(57) Abstract

Bifunctional chelating agents are designed to sequester certain radioactive metals, such as gallium (III) isotopes, and to provide a means for covalently attaching these radionuclides to macromolecules, such as monoclonal antibodies. These chelating agents may be used in various therapeutic and diagnostic methods, such as in radioimaging and positron emission tomography.

DERIVATIZED TRIS-CATECHOL CHELATING AGENTS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to novel "bifunctional" chelating agents. More specifically, the present invention relates to bifunctional chelating agents which are designed to sequester certain radioactive metals and to provide a means for covalently attaching these radionuclides to a macromolecule, such as an antibody. The invention further relates to methods for preparing these compounds as well as methods of using these compounds in radioimmunoimaging, positron emission tomography and *in vivo* treatment. The present invention further relates to these compounds attached to antibodies.

Description of Related Art

Effective therapeutic methods for the treatment of cellular disorders such as cancer have been the object of intensive research. Conventional therapy employs surgery, radiation and chemotherapy. Each of these methods suffers a serious drawback in that it is not highly selective between healthy and cancerous cells. In order to be effective, these methods kill or remove large amounts of healthy tissue. Furthermore, chemotherapy adversely affects the immune system so that death or serious illness often arises from fungal, bacterial or viral infections.

The development of monoclonal antibodies has opened the possibility of selectively delivering therapeutic agents or diagnostic agents to specific target cells. Monoclonal antibodies are immunoglobulins of well-defined chemical structure. A characteristic feature of monoclonal antibodies is reproducibility of function and high specificity.

provide a means for covalently attaching the radionuclides to macromolecules.

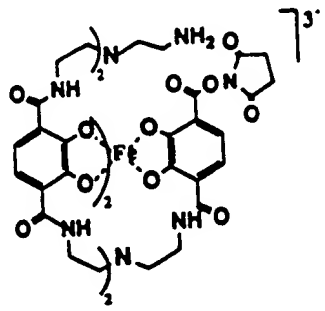
It is another object of the present invention to provide a method for preparing bifunctional chelating agents.

It is a further object of the present invention to provide diagnostic and therapeutic techniques which employ these bifunctional chelating agents in the form of radiometal chelate conjugated monoclonal antibodies.

The foregoing objects and others are accomplished in accordance with the present invention, generally speaking, by providing bifunctional chelating agents having a tris-catechol structure and a method for preparing the same, wherein these agents are useful for sequestering radioactive metals (radionuclides) and for providing a means for covalently attaching these radionuclides to a macromolecule, such as an antibody. The present invention further encompasses therapeutic and diagnostic techniques which employ the bifunctional chelating agents in the form of radiometal chelate conjugated monoclonal antibodies.

Further scope of the applicability of the present invention will become apparent from the detailed description and drawings provided below. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The above chelating agents are prepared in accordance with the present invention by derivatizing an intermediate 3 (below) in accordance with the synthetic procedures described for synthesizing macrobicyclic tris-catechol ligand in McMurry et al, J. Am. Chem. Soc., Vol. 109, pp. 3451-3453 (1987).



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The synthetic scheme for the chelating agents of the present invention is summarized below.

As shown in the scheme above, the disuccinimido-2,3-dibenzyloxyterephthalate **2** is reacted with two equivalents of the ligand tris(2-aminoethyl)amine (TREN) in the presence of iron (III) to give the metal complex **3**. This reactive intermediate may be reacted with 1-amino-2-(p-NO_2-Benzyl)ethane to give the derivatized complexes **4a,b**. The reactive alkylamine is then protected with acetic anhydride to give **5a, b** and then the aromatic amine reduced to provide the aniline metal complex derivative **6** that is subsequently demetalated to provide the amino ligand **1** which may be reacted with thiophosgene to give the isothiocyanate ligand **1a**. Both **1, 1a** are useful for linkage of the ligand to proteins, such as antibodies, by carbohydrate modification methods for **1** and by direct reaction with amino acid residues with **1a**.

The present invention employs metal chelate conjugated monoclonal antibodies for diagnostic and therapeutic techniques, particularly in vivo. The metal may be radioactive, exhibit fluorogenic properties, exhibit paramagnetic properties or the like.

Monoclonal antibodies are immunoglobulins of well-defined chemical structure, in contrast to polyclonal antibodies which are heterogeneous mixtures. Reproducibility of function cannot be controlled for either polyclonal or autologous antibodies, whereas unaltered function is characteristic to monoclonal antibodies. Experimental techniques for obtaining monoclonal antibodies have been extensively discussed. A useful text is Monoclonal Antibodies (R.H. Kennett, T.J. McKearn & K.B. Bechtol eds. 1980). See also Koprowski et al. U.S. Patent 4,196,265 which is incorporated herein by reference. Any monoclonal

Only enough radiation for the target cells need be employed. In addition, radiometal chelates generally are cleared rapidly from the body should the conjugated antibody be disrupted. The isotope can be short-lived and the affinity constant by which the isotope that is retained in the chelate is very high resulting in a stably bound metal. Finally, since the amount of radiometal employed is minimized, the radiation hazard to persons preparing and administering the radiometal chelate conjugated antibody is also minimized.

Because of the properties of the radiometal chelate conjugated monoclonal antibody employed by the present invention, tissue damage or whole body dose during therapy are markedly reduced as compared to that from presently employed methods of radiation therapy such as isotope implants, external radiation therapy such as isotope implants, external radiation therapy, and immunoradiotherapy employing iodine-131 labeled polyclonal or autologous antibodies. Additionally, both biological and physical half-lives of the targeting radiobiological may now be controlled, minimizing whole body radiation effects. Since radiation is targeted specifically to cell types (e.g. neoplastic cells), a therapeutic dose is delivered specifically to malignant cells, either localized or metastasized. The ability of radiometal chelate conjugated monoclonal antibody to provide an effective dose or therapeutic radiation specifically to metastasized cells is also unique and singularly useful for cancer therapy.

In one of its particularly preferred aspects, the present invention employs the metal chelate conjugated monoclonal antibody containing a positron emitting radiometal to treat cellular disorders. It is desirable

these forms of emission, or properties (optical or magnetic), available in the art.

The metal chelate conjugated antibodies of this invention can be administered in vivo in any suitable pharmaceutical carrier. As noted earlier, a physiologic normal saline solution can appropriately be employed. Often the carrier will include a minor amount of carrier protein such as human serum albumin to stabilize the antibody. The concentration of metal chelate conjugated antibodies within the solution will be a matter of choice. Levels of about 0.5 mg per ml are readily attainable but the concentrations may vary considerably depending upon the specifics of any given application. Appropriate concentrations of biologically active materials in a carrier are routinely determined in the art.

The effective dose of radiation or metal content to be utilized for any application will also depend upon the particulars of that application. In treating tumors, for example, the dose will depend, inter alia, upon tumor burden, accessibility and the like. Somewhat similarly, the use of metal chelate conjugated antibodies for diagnostic purposes will depend, inter alia, upon the sensing apparatus employed, the location of the site to be examined and the like. In the event that the patient has circulating antigen in addition to those located at the site, the circulating antigens can be removed prior to the treatment. Such removal of antigens can be removed prior to treatment. Such removal of antigens can be accomplished, for example, by the use of unlabeled antibodies, or by plasmapheresis in which the patient's serum is treated to remove antigens.

added 3-(para-nitrophenyl)propylamine hydrochloride (0.7 g, 3.2 mmol) and triethylamine (1 ml, ca, 7 mmol). The solution was stirred for 12 hours (HPLC retention time of product, 11.19 minutes), and the DMF evaporated to dryness. Aqueous ammonium acetate (0.01 M, 350 ml) was added and the pH adjusted to 9.7 with NH_4OH. The solution was stirred 12 hours and the insoluble materials removed by filtration. The pH of the filtrate was adjusted to 6.8 with glacial acetic acid and the volume diluted to 400 ml with 0.01 M $AcONH_4$. Purification was achieved by HPLC using a Waters Delta Prep and a Waters Delta Pak preparative C-18 reverse phase column (30 x 300 mm, 15 micro spherical packing, 100 A pore size) with a mobile phase of A = H_2O and B = MeOH (both 0.01 M $AcONH_4$). In a typical run, 10 ml of the above solution (D) was loaded and the products eluted with a 0-100% B (10%/min) gradient at 40 ml/min. The fraction eluting between 8.7 - 9.7 minutes was collected. The procedure was repeated until all crude material was purified. The aqueous solutions of product were evaporated to dryness and redissolved in ca. 125 ml H_2O. The solution was acidified to pH 3.05 with glacial acetic acid, resulting in a blackish precipitate (the neutral ferric complex), which was collected on a medium frit and washed with ca. 60 ml H_2O and dried to give 0.988g (1.03 mmol, 45%). A summary of the HPLC results is provided below in Table 1.

addition of Et_3N (.41 ml, ca. .3g, 2.9 mmol, 6 equiv.) was added and the reaction stirred 75 minutes. HPLC (conditions #2, Gilson) show no starting material (RT 11.07 min) and a single product (RT 10.37 min). The DMF was evaporated to give an oil, to which 10 ml H_2O was added. Glacial acetic acid was added to precipitate the ferric complex, which was collected on a fine glass frit, washed with water and dried to give 0.45 g (0.43 mmol, 87%).

Preparation of Et_3NH salt of acetamide 5b

Neutral complex **5a** (0.15 g, .14 mmol) was suspended in 5 ml CH_3OH and stirred while triethylamine (0.12 ml, 0.086 g, .85 mmol) was added. The resulting burgundy solution was evaporated to dryness, redissolved in 20 ml methanol and again evaporated to dryness. The solid was taken up in ca. 1 ml methanol, precipitated by the addition of diethyl ether, and collected on a fine glass frit. The solid was vacuum dried to give 0.19 g (.14 mmol, 95%) of the triethylammonium salt 5b.

Elemental Analysis: Calc for $[Fe(C_{47}H_5ON_{10}O_{15}]_3[C_6H_{16}N+]_3\ 3H_2O$:C,57.51; H,7.28; N,13.41; Fe,4.11. Found: C,55.31; H,7.43; N,12.90; Fe,3.96.

Reduction to aniline and demetallation to give 1

Neutral ferric complex **5a** (0.4 g, .38 mmol) was suspended in 10 ml H_2O and solubilized by the addition of NaOH (1.2 ml 1M NaOH, 1.1 mmol). The pH of the solution was adjusted to 7.4, and was then transferred via syringe to a 50 ml 3 neck flask containing 250 mg 10% Pd/C (saturated with H_2). Hydrogenation at atmospheric pressure was complete in 7-8 hours, as evidenced by the analytical HPLC (conditions #2) which showed the complete conversion of starting material (RT 10.4) to a single

supernatent removed, and the solid washed with distilled water. After vacuum drying, 25 mg of product was obtained. IR (nujol) 3300 (m), 2080(s).

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

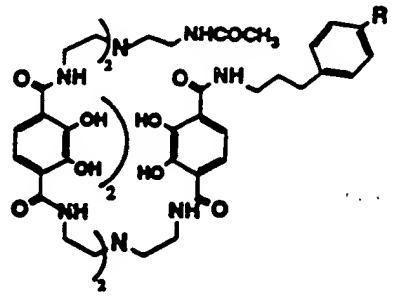
wherein R is $-NH_2$ or -NCS, said compound being in the form of a radiometal chelate conjugated macromolecule or antibody; and a pharmaceutically acceptable excipient.

4. Use of a solution of the radiometal chelate conjugated antibodies of claim 3 specific for a target cell for an in vivo diagnostic method for the treatment of cellular disorders wherein said solution is introduced into body fluid.

5. Use of a solution of the radiometal chelate conjugated antibodies of claim 3 for an in vitro diagnostic method which comprises introducing into a test medium said solution and quantifying the specifically bound portion of said conjugate.

6. Use according to claim 5 wherein quantifying is conducted by using radioimmunoimaging or positron emission tomography.

7. A method for preparing final product compounds of the formula:



wherein R is $-NH_2$ or -NCS,

which method comprises the steps of:

reacting disuccinomido-2,3-dibenzyloxyterephthalate with tris(2-aminoethyl)amine in the presence of iron (III) to form an intermediate metal complex;

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09153

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT CL.(5): A61K 49/02 C07D 245/00 G01N 23/00
U.S. CL.: 424/1.1 546/460 436/57

II FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/1.1 540/460 436/57,804 534/10,14

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Journal of American Chemical Society, Volume 109, No. 11, issued November 1987 (USA), T.J. MCMURRAY ET. AL., 'Template and Stepwise Syntheses of a Macrobicyclic Catechoylamide Ferric Ion Sequestering Agent,' see pages 3451-3453, especially page 3452.	7
A	US, A, 4,732,974 (NICHOLOTTI ET. AL.) 22 MARCH 1988	1,7

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 FEBRUARY 1992	12 MAR 1992
International Searching Authority	**Signature of Authorized Officer**
IPEA/US	JOHN S. MAPLES

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No

Class 424, subclass 1.1.

IV. Claims 5-6, drawn to a method of in vitro use, classified in Class 436, subclass 57.